2266

SHORT COMMUNICATIONS

The glucose oxidase of honey. III. Kinetics and stoichiometry of the reaction

sc 63092

Previous studies[1] on the glucose oxidase (β-D-glucose:O_2 oxidoreductase, EC 1.1.3.4) of honey revealed an unusually high substrate-concentration requirement. This property has now been investigated in greater detail and a Michaelis constant determined. In addition, the reaction was shown to be stoichiometric and of zero order.

The glucose oxidase was prepared as previously described[1,2]. It had a specific activity of 224 units/mg (see ref. 1 for definition of units). The substrate was a solution of equilibrium glucose made from α-D-glucose (J. T. Baker Chemical Co.*). Catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) and sodium pyruvate were obtained from Sigma Chemical Co. All experiments were run at 37° using standard Warburg manometric procedures.

The effect of substrate concentration on velocity of the reaction was studied by keeping the enzyme concentration constant (223 units) and varying the substrate concentration over a range from 0.1 to 4.0 M. A typical progress curve at one of these concentrations is shown in Fig. 1. The velocity-substrate curve, obtained from the initial velocity at each concentration, is shown in Fig. 2. The optimum substrate concentration is in the area of 2.7 M. Another series of experiments at twice the

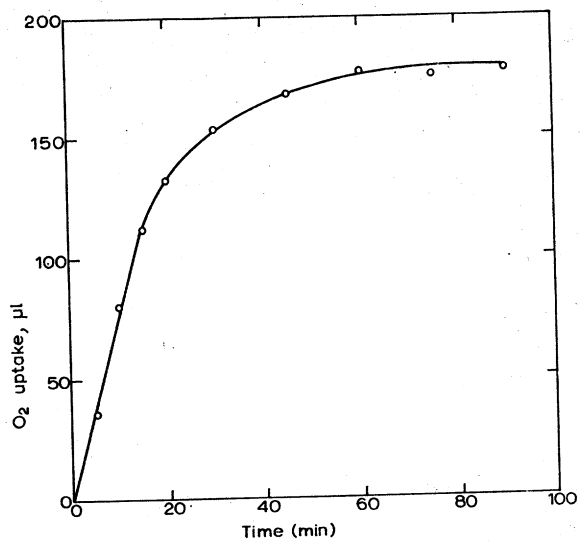

Fig. 1. Progress curve for honey glucose oxidase. The complete system contained 2.7 ml 3.5 M glucose in 0.2 M sodium phosphate (pH 6.1), 0.5 ml same buffer, 0.1 ml (800 Sigma units) catalase and 0.1 ml 95% ethanol in the mainspace; 0.1 ml (223 units) honey glucose oxidase in side-arm sac; 0.2 ml 10% KOH in center well. Vessels were gassed with 100% O_2. Blanks were run without enzyme, without substrate.

* Mention of company or trade names does not imply endorsement by the Department over others of a similar nature not named.

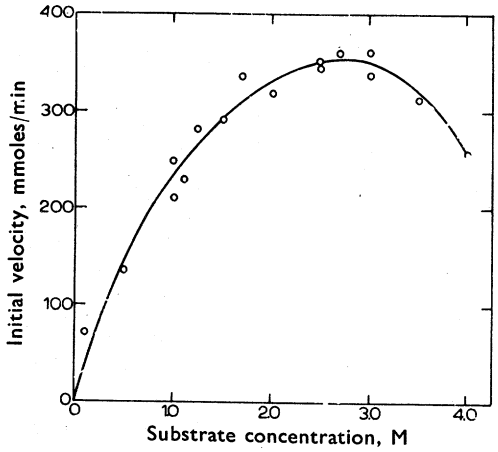

Fig. 2. Effect of substrate concentration on velocity. Experimental conditions were the same as in Fig. 1 except that glucose concentration was varied from 0.1 to 4.0 M. A 4.5 M glucose solution was used to obtain the high final concentrations. The 0.2 M sodium phosphate buffer was added as needed to give a total volume of 3.7 ml.

enzyme concentration (446 units) resulted in a similar velocity-substrate curve again having an optimum at 2.5 to 3.0 M. Subjecting both sets of data to LINEWEAVER AND BURK[3] plots and drawing lines statistically fitted by the method of least squares, Michaelis constants (K_m) of 1.49 and 1.70 M, respectively, were obtained. Fig. 3 illustrates the former of these plots. The statistical data indicated that the straight-

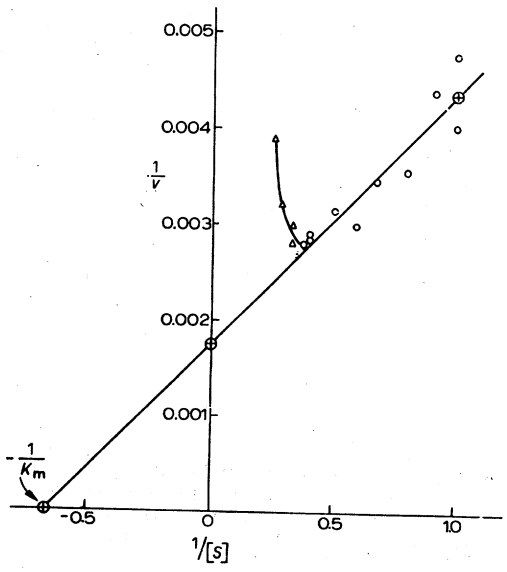

Fig. 3. Lineweaver–Burk plot. Points were derived from those in Fig. 2; v, initial velocity; $[S]$, substrate concentration; K_m, Michaelis constant; ○, points included in statistical analysis; △, points not included in statistical analysis since beyond optimum concentration; ⊕, points derived from statistics and used to locate line.

line plots were highly significant ($P = 0.99$*), showing a high correlation (r** = 0.96, 0.94, respectively) between $1/v$ and $1/[S]$, and that the K_m values were definitely greater than 1.0.

Since the above optimum substrate concentration did not correspond with the value of 1.5 M found previously[1], the use of catalase and ethanol in the system (as recommended by KEILIN AND HARTREE[4]) in the present experiments was investigated. No effect on the initial velocity was found except at the extremely low substrate level of 0.1 M (where the mold enzyme of KEILIN AND HARTREE[4] is active). The catalase and ethanol served only to sustain the reaction (presumably by removal of H_2O_2). Substitution of sodium pyruvate[5] for catalase and ethanol gave similar results.

Another series of experiments at an enzyme level of 223 units was run without catalase and ethanol. The velocity-substrate curve had an optimum at 2.5–2.7 M. The Lineweaver–Burk plot, again handled statistically, resulted in a highly significant ($P = 0.99$, $r = 0.966$) straight-line relationship of $1/v$ and $1/[S]$ and a $K_m = 1.47$ M.

The present optimum substrate concentration of about 2.7 M, having been derived repeatedly under varying conditions in this more extensive study, is considered more reliable than the 1.5 M value obtained previously[1]. Although 2.7 M is an unusually high substrate requirement, it is not inconceivable. The glucose oxidase found in the bee (from which the honey enzyme may have originated[1]) requires a glucose concentration of about 2 M (ref. 6). It may also be noted that the value of 2.7 M is in terms of equilibrium glucose, whereas in terms of the more specific substrate, β-D-glucose[2], the optimum is actually 1.8 M.

The high Michaelis constant (average $K_m = 1.55$ M) is in contrast with a $K_m = 0.0042$ M reported for the mold glucose oxidase[7], but is comparable to a $K_m = 0.63$ M derived from the data on the bee enzyme[6].

The linearity of the initial portion of the progress curve (Fig. 1) indicates that the reaction follows a typical zero order during this period. In the course of such a

TABLE I

MANOMETRIC DETERMINATION OF STOICHIOMETRY

The complete system contained 2.5 ml 3.5 M glucose in 0.2 M sodium phosphate (pH 6.1), 0.8 ml same buffer and 0.1 ml sodium pyruvate (35 mg/ml) in the main-space; 0.1 ml (223 units) honey glucose oxidase in one side-arm sac; 0.1 ml 3 N HCl or 0.1 ml H_2O in another side-arm sac; 0.1 ml 10% KOH in center well of vessels containing no HCl, 0.1 ml H_2O in all others. Blanks were run without enzyme, without substrate. All vessels were gassed with 100% O_2. Acid was tipped in at t_0 for initial CO_2 in one vessel, at t_{30} for total CO_2 in all CO_2 vessels.

	Total O_2 uptake	*Total CO_2 output*		
		Sample 1	*Sample 2*	*Average*
μl	190.2	191	189	190
μmoles	8.5	—	—	8.5

* The probability that the relationship is highly significant is equal to 0.99.
** r is the correlation coefficient.

reaction only about 0.08% of the substrate is oxidized in 60 min. Since the substrate concentration never becomes a limiting factor, the reaction order must remain zero. The observed decrease in slope of the progress curve after the initial portion presumably is due to inhibitory processes.

The stoichiometry of the reaction was established by making use of the long-known decarboxylation of pyruvic acid by H_2O_2 (ref. 5). In the balanced reactions:

$$\underset{\text{glucose}}{C_6H_{12}O_6} + O_2 + H_2O \rightarrow \underset{\text{gluconolactone}}{C_6H_{12}O_7} + H_2O_2 \quad (1)$$

$$H_2O_2 + CH_3COCOOH \rightarrow CH_3COOH + H_2O + CO_2 \uparrow \quad (2)$$

1 mole O_2 = 1 mole H_2O_2 = 1 mole CO_2. By use of the Warburg "Direct Method for CO_2" (ref. 8), it was shown (Table I) that this relationship holds, indicating that Reaction 1 is stoichiometric.

From the evidence presented we conclude that the optimum substrate concentration of the honey glucose oxidase is 2.7 M and the Michaelis constant is 1.55 M. The reaction follows a zero order and is stoichiometric.

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